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ALCOHOLYSIS OF ε-DECALACTONE WITH POLYETHYLENE GLYCOL-MODIFIED LIPASE IN 1,1,1-TRICHLOROETHANE

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SUMMARY: Lipase from *Pseudomonas cepacia* was modified with 2,4-bis[*O*-methoxypoly(ethylene glycol)]-6-chloro-*s*-triazine, activated PEG_2, to form PEG-lipase. The PEG-lipase is soluble and active in organic solvents. It catalyzes alcoholysis of racemic ε-decalactone with ethanol in 1,1,1-trichloroethane to form (*R*)-hydroxydecanoic acid ethyl ester. No alcoholysis of (*S*)-decalactone takes place. These results were discussed in relation to carbon number of *n*-alcohol, optimum temperature and comparison with modified and non-modified lipases.

In 1984, it was found that enzymes coupled with a polyethylene glycol derivative(PEG) become soluble and exhibit the enzymic activity in organic solvents, such as benzene, toluene and 1,1,1-trichloroethane(1). The PEG-lipase catalyzes effectively the reverse reactions of hydrolysis, ester synthesis and ester exchange reactions(2). In fact, a linear polymer of 10-hydroxydecanoic acid was synthesized with PEG-lipase in benzene(3) and a lactone was also synthesized from 16-hydroxyhexadecanoic acid ethyl ester(4). They are inter- and intra-molecular esterifications of each substrate, respectively. PEG-lipase from *Pseudomonas fragi* 22.39B recognizes the chirality of alcohols and catalyzes preferentially ester synthesis reactions from an (*R*)-secondary alcohol and a fatty acid(5).

Lactones, bioactive substances, are widely utilized in the fields of foods, perfumes and pharmaceuticals. Although racemic lactones can be synthesized, chiral lactones are hardly obtained by organic reactions.

The present paper deals with the alcoholysis of ε-decalactone with PEG-lipase in 1,1,1-trichloroethane in the hope of obtaining a chiral lactone.

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Materials and Methods

Lipase from *Pseudomonas cepacia* was kindly donated from Amano Pharmaceuticals Co. Ltd.(Nagoya, Japan) ε-Decalactone was purchased from Soda Aromatic Co. Ltd.(Tokyo, Japan) 2,4-Bis[*O*-methoxypoly(ethylene glycol)]-6-*s*-triazine, activated PEG_2, was synthesized by the method of Ono *et al.*(6) Other reagents were of analytical grade.

Preparation of PEG-lipase: PEG-lipase was prepared as described previously(2). The degree of modification of amino groups in the lipase molecule was 70% and the hydrolytic activity in emulsified olive oil was 600 units/mg, which was determined by the method of Habeeb(7) and Watanabe *et al.*(8), respectively.

Alcoholysis of ε-decalactone: To 0.1 ml of 1,1,1-trichloroethane containing racemic ε-decalactone(100 mM) and a *n*-alcohol(1 M) were added 0.9 ml of PEG-lipase(0.02 mM) dissolved in the same solvent. The reaction mixture was incubated at a temperature ranging from 15 °C to 65 °C. Amounts of the substrate and the products were analyzed with Shimadzu GC-14A gas chromatography(Kyoto, Japan) equipped with a flame ionization detector(FID). A capillary column of Astec Chiraldex B-PH (20 m x 0.25 mm ID, 0.125 μm in film thickness, Madras, OR) was used. The column temperature was increased from 90 °C to 150 °C in linear gradients of 0.4 °C/min for 35 min and 4 °C/min for 11.5 min. *n*-Hexadecane was used as the internal standard.

Results and Discussion

As a preliminary experiment, it was found that PEG-lipase catalyzes the alcoholysis of ε-decalactone with ethanol in 1,1,1-trichloroethane to form 6-hydroxydecanoic acid ethyl ester, HDE. Figure 1 shows the chemical formula of the reaction process

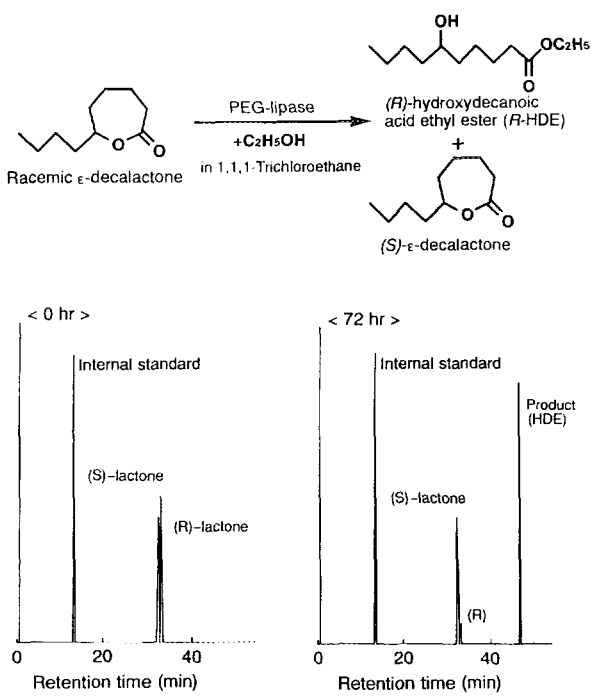
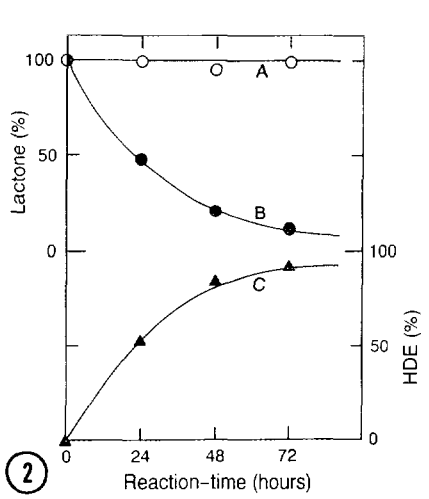


Fig. 1. Alcoholysis of ε-decalactone with PEG-lipase in 1,1,1-trichloroethane and the chromatographic pattern obtained for the alcoholisis reaction for 0 and 72 hr at 65 °C.



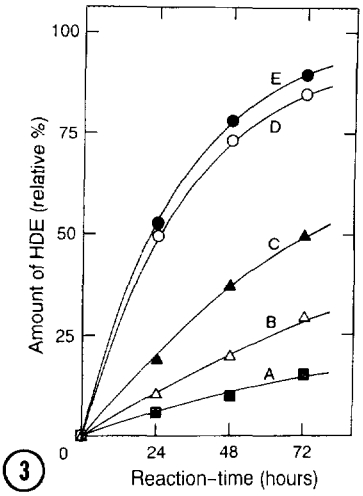


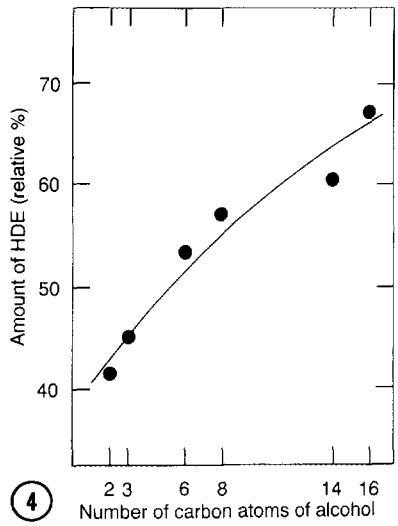
Fig. 2. The time-course of alcoholysis of ε-decalactone with ethanol for 72 hr and at 65 °C using PEG-lipase. **Curves A and B**; Amounts of (*S*)-ε-decalactone and (*R*)-ε-decalactone, respectively. **Curve C**; Amount of (R)-hydroxydecanoic acid ethyl ester.

Fig. 3. The effect of reaction-temperature on the alcoholysis of ε-decalactone with ethanol in 1,1,1-trichloroethane for 72 hr, using PEG-lipase. **Curves A, B, C, D and E**: 15, 25, 35, 50 and 65 °C, respectively.

together with the chromatographic pattern obtained for the alcoholysis reaction. As is seen in the pattern, the peak of (*R*)-lactone lowers and that of HDE appears newly for 72 hr-incubation. No change of peak area of (*S*)-lactone is observed for the incubation. The product formed in this reaction is, therefore, (*R*)-6-hydroxydecanoic acid ethyl ester, (*R*)-HDE.

Figure 2 represents the time-course of the alcoholysis of ε-decalactone with ethanol in 1,1,1-trichloroethane at 65 °C using PEG-lipase. The amount of (*S*)-ε-decalactone is not changed during the reaction-time at all(curve A), while that of (*R*)-ε-decalactone is sharply decreased with reaction-time and tends to approach a constant level of zero(curve B). On the other hand, (*R*)-6-hydroxydecanoic acid ethyl ester sharply appears with time and approaches a constant level of 100%(curve C). These results indicate that (*R*)-ε-decalactone is preferentially changed to (*R*)-6-hydroxydecanoic acid ethyl ester by alcoholysis using PEG-lipase.

Figure 3 shows the temperature-dependent curves obtained for the alcoholysis of ε-decalactone with ethanol with PEG-lipase in 1,1,1-trichloroethane at 15 °C, 25 °C, 35 °C, 50 °C and 65 °C(curves A, B, C, D and E, respectively). The rate of HDE-formation is enhanced by increasing the temperature ranging from 15 °C to 65 °C. Above 80 °C, the rate of the alcoholysis with PEG-lipase is decreased by heat-denaturation of the enzyme. The optimum temperature of hydrolysis of esters with non-modified lipase is 50 °C in an aqueous emulsified system. It is interested to note that the optimum temperature in the alcoholysis with PEG-lipase in 1,1,1-



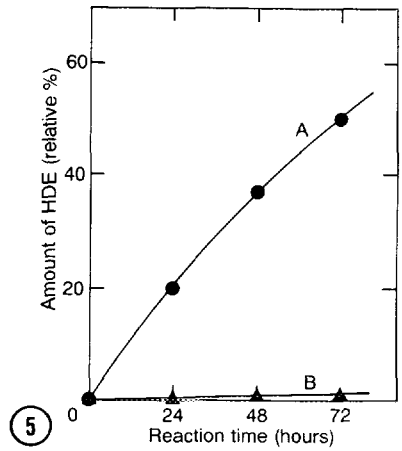


Fig. 4. The effect of carbon number of alcohols on the alcoholysis of ε-decalactone with PEG-lipase at 25 °C for 72 hr.

Fig. 5. The comparison between PEG-lipase and non-modified lipase on the alcoholysis of ε-decalactone with ethanol in 1,1,1-trichloroethane at 35 °C. **Curves A and B**: PEG-lipase and non-modified lipase, respectively. The hydrolytic activities of non-modified and modified-lipases are the same as (approximately 480 units/ml) in emulsified olive oil as substrate.

trichloroethane is elevated by 65 °C in comparison with 50 °C for the hydrolysis of esters with non-modified lipase.

In the alcoholysis of ε-decalactone with PEG-lipase in 1,1,1-trichloroethane, it was tested about the effect of carbon number in alcohols on the formation of hydroxydecanoic acid alkyl esters at 25 °C. The results are shown in Fig. 4. Increasing the carbon number of alcohols from ethanol(C_2) to hexadecanol(C_{16}) gives rise to the high yield of the products for 72 hr-incubation, suggesting that PEG-lipase recognizes the carbon chains of alcohol substrates in 1,1,1-trichloroethane. This phenomenon is compatible with that the Km value of primary alcohols on ester synthesis with PEG-lipase in benzene enhanced by increasing the carbon number of alcohol(9).

The alcoholysis activity of ε-decalactone with PEG-lipase in 1,1,1-trichloroethane was compared with that of non-modified lipase in the same solvent, which is shown in Fig. 5. PEG-lipase catalyzes effectively the alcoholysis reaction(curve A) and its rate at alcoholysis is 20 times higher than that with non-modified lipase(curve B). From the results obtained above, it can be concluded that the modification of lipase with an amphipathitic synthetic macromolecule, polyethylene glycol derivative, has an advantage to alcoholysis as well as ester synthesis and ester exchange reactions in organic solvents(10). Further, it was found that assymetric alcoholysis of ε-decalactone proceeds in organic solvent with PEG-lipase. This finding offers a promissing prospect of resolving racemic lactones for flavors or medicines such as macrolide antibiotics.

References

1. Takahashi, K., Nishimura, H., Yoshimoto, T., Saito, Y. and Inada, Y. (1984) Biochem. Biophys. Res. Commun. **121**, 261–265.
2. Inada, Y., Nishimura, H., Takahashi, K., Yoshimoto, T., Saha, A. R. and Saito, Y. (1984) Biochem. Biophys. Res. Commun. **122**, 845–850.
3. Ajima, A., Yoshimoto, T., Takahashi, K., Tamaura, Y., Saito, Y. and Inada, Y. (1985) Biotechnol. Lett. **7**, 303–306.
4. Kodera, Y., Furukawa, M., Yokoi, M., Kuno, H., Matsushita, H. and Inada, Y. J. Biotechnol.,in press.
5. Kikkawa, S., Takahashi, K., Katada, T. and Inada, Y. (1989) Biochem. Inter. **19**, 1125–1131.
6. Ono, K., Kai, Y., Maeda, H., Samizo, F., Sakurai, K., Nishimura, H. and Inada, Y. (1991) J. Biomater. Sci. Polymer Edn. **2**, 61–65.
7. Habeeb, A. F. S. A. (1966) Anal. Biochem. **14**, 328–336.
8. Watanabe, N., Ota, Y., Minoda, Y. and Yamada, K. (1977) Agric. Biol. Chem. **41**, 1353–1358.
9. Takahashi, K., Yoshimoto, T., Ajima, A., Tamaura, Y. and Inada, Y. (1984) Enzyme **32**, 235–240.
10. Inada, Y., Matsushima, A., Takahashi, K. and Saito, Y., (1990) Biocatalysis **3**, 317–328.